

Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial

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A prospective randomized study comparing single embryo transfer with double embryo transfer after in-vitro fertilization or intracytoplasmic sperm injection (IVF/ICSI) was carried out. First, top quality embryo characteristics were delineated by retrospectively analysing embryos resulting in ongoing twins after double embryo transfer. A top quality embryo was characterized by the presence of 4 or 5 blastomeres at day 2 and at least 7 blastomeres on day 3 after insemination, the absence of multinucleated blastomeres and <20% cellular fragments on day 2 and day 3 after fertilization. Using these criteria, a prospective study was conducted in women <34 years of age, who started their first IVF/ICSI cycle. Of 194 eligible patients, 110 agreed to participate of whom 53 produced at least two top quality embryos and were prospectively randomized. In all, 26 single embryo transfers resulted in 17 conceptions, 14 clinical and 10 ongoing pregnancies [implantation rate (IR) = 42.3%; ongoing pregnancy rate (OPR) = 38.5%] with one monozygotic twin; 27 double embryo transfers resulted in 20 ongoing conceptions with six (30%) twins (IR = 48.1%; OPR = 74%). We conclude that by using single embryo transfer and strict embryo criteria, an OPR similar to that in normal fertile couples can be achieved after IVF/ICSI, while limiting the dizygotic twin pregnancy rate to its natural incidence of <1% of all ongoing pregnancies.

Key words: IVF/single embryo transfer/twin pregnancy

Introduction

The increasing success of in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) is complicated by an increased risk of twin and higher order multiple pregnancies. The debate on how to determine the number of embryos at embryo transfer in order to obtain an acceptable pregnancy rate while truly minimizing the risk for twin or higher order multiple pregnancy has been extensive (Seoud *et al.*, 1992; Baldwin, 1994; Jones, 1995; Bronson, 1997; Bustillo, 1997; Faber, 1997; Van Blerkom, 1997; Hu *et al.*, 1998; Templeton and Morris, 1998). The frequency of multiple pregnancies is correlated with the number of high quality embryos transferred

(Staessen *et al.*, 1992; Roseboom *et al.*, 1995; Yaron *et al.*, 1997). There has been a considerable increase in multiple births all over the world. Both obstetric and neonatal complications are increased in all multiple gestations, resulting in higher medical costs and human suffering. Children from higher order pregnancies are at a greater risk than twins, but, since the latter are more frequent, they constitute the foremost and largest problem.

Initially, multiple pregnancy was tolerated both by physicians and patients because it was a compensation for the relatively low ongoing pregnancy rate of IVF. The establishment of ICSI (Van Steirteghem *et al.*, 1993a,b), including the use of surgically retrieved spermatozoa, was a breakthrough for male infertility, and boosted the initial habit of replacing three or more embryos, especially in countries without a social security system. However, nowadays an overall pregnancy rate of 30–40% per started cycle is obtained in many centres, even after replacement of no more than two embryos. This increased efficiency of IVF/ICSI is due to several factors, such as the younger age of patients treated with ICSI, improved culture media, improved identification of viable embryos and an improved transfer procedure. A general climate has resulted in which high order multiple pregnancies are no longer considered good clinical practice. Nevertheless, the balance between the need for a high success rate and a low complication rate remains very delicate and is determined by a number of opposing forces. Arguments in favour of a high number of embryos to be transferred are the high physical, emotional and financial cost of treatment for the patients, competition among neighbouring centres to obtain a high success rate, a general underestimation of the complications of multiple pregnancy by the patients, a positive desire for twin pregnancy by many patients and waiting lists for treatment in some countries. Arguments in favour of a low number of embryos to be transferred are the strongly increased maternal and perinatal risks of multifetal pregnancy, the fact that prenatal diagnosis can be performed more easily in singletons, increasing social and political pressure and a decreased need for multifetal pregnancy reduction.

A number of strategies have been proposed which try to preserve a high pregnancy rate while ‘minimizing’ multiple pregnancies. Almost all of these strategies have aimed at avoiding triplets but not twins and they have all failed to bring the twin rate down substantially, let alone in the vicinity of its natural frequency of ~1%.

Reduction of a triplet or a higher-order multiple pregnancy to a twin pregnancy has been proposed as a solution to reduce perinatal mortality and morbidity and is available in a number of countries. Although this technique does in fact reduce the

risk of triplets to that of twins, it has the disadvantage of creating minor and occasional major obstetric and psychological problems. Moreover, systematic reduction of a twin to a singleton will not be accepted by the public at large as an ethically acceptable solution to manage the complications and hazards of twins.

Thus, it was decided to conduct a prospective randomized trial comparing single embryo transfer with double embryo transfer, at a time when the overall ongoing pregnancy rate in our centre had remained stable above 35% for a period of more than 1 year with >30% of twin pregnancies. The aim of the study was to obtain data on the implantation rate and the (multiple) pregnancy rate after single embryo transfer and double embryo transfer, when compared in a prospective manner.

Materials and methods

Patients

Identification of patients at risk—retrospective analysis

It is not advisable to transfer only one embryo to all patients participating in an IVF/ICSI programme. Older patients, patients who have had several unsuccessful treatment cycles, patients who smoke, patients who show predominantly poor quality embryos (Magli *et al.*, 1998) and perhaps other groups of patients are known to have a decreased implantation rate.

Two questions arose before conducting the prospective study: (i) first, what were the morphological characteristics of top quality embryos, i.e. embryos with a very high implantation potential; (ii) what was the clinical outcome in patients who received two such top quality embryos at transfer? The answers were obtained by reviewing retrospectively a continuous series of 400 IVF/ICSI cycles immediately prior to the start of the prospective study (Van Royen *et al.*, 1999). In brief, top quality embryos were defined as exhibiting all of the following characteristics: 4 or 5 blastomeres on day 2 and at least 7 blastomeres on day 3 after fertilization, absence of multinucleated blastomeres and <20% of fragments on day 2 and day 3 after fertilization.

Study population during the prospective study

A total of 327 patients completed a total of 545 IVF/ICSI-embryo transfer cycles during the prospective study period which ran from November 1997 until May 1999. The mean age of the women was 31.9 (range: 22–44) years with an average duration of infertility of 3.5 (range: 1–11) years. We previously ascertained that the success rate in all infertility indications in our centre was similar. Patients with semen dysfunction were treated with standard IVF or ICSI, depending on the semen characteristics of the treatment sample on the day of oocyte retrieval.

Study design

For ethical reasons, we included only patients in their first IVF/ICSI cycle and who were <34 years of age. Eligible patients were counselled extensively regarding the risks of multiple pregnancy and were thoroughly informed regarding the strict study design. They were offered the opportunity to participate to the study but were clearly informed that they could refuse to take part in the study without any disadvantage for further treatment. In this latter case, 'standard' treatment was applied consisting of a transfer of the two best embryos if two were available. In order to be included in the randomization, the patient had to agree to participate in the study and she had to have at least two top quality embryos at the time of

embryo transfer. Randomization took place at the time of embryo transfer using external concealment. Patients were told that all supernumerary embryos would be frozen and that the study was limited to the first treatment cycle. Patients who declined to participate and patients who had agreed but who failed to produce two top quality embryos were always given the two best quality embryos except if only one embryo was available. Due to the counselling, some patients elected to have a single embryo transfer (elective single embryo transfer) of a top quality embryo in all cases.

In total, 327 patients participated in the IVF/ICSI programme during the study period, completing a total of 545 cycles. Of these, 194 (35.6%) were first treatment cycles in women <34 years of age and were therefore eligible for the study. Of these women, 110 agreed to the study protocol and were therefore recruited. Of those agreeing to participate in the study, 53 fulfilled the study inclusion criteria, i.e. they produced at least two top quality embryos and 57 did not. The other 84 patients either requested an elective single embryo transfer ($n = 17$) or did not agree to participate ($n = 67$). The number of patients included in the study was thus lower than the number recruited, which was lower than the potentially eligible target group, which was by itself much smaller than the whole population. There were six major groups of cycles to consider: group A ($n = 26$): patients (and cycles) randomized to single embryo transfer; group B ($n = 27$): patients (and cycles) randomized to double embryo transfer; group C ($n = 57$): patients (and cycles) complying with the inclusion criteria but who failed to produce two top quality embryos so that randomization was impossible; group D ($n = 17$): patients (and cycles) requesting elective single embryo transfer (11 received one top quality embryo single embryo transfer, six did not); group E ($n = 67$): patients (and cycles) not wanting to participate in the study and who received standard treatment; and group F ($n = 133$): patients not eligible to participate in the study (all other IVF/ICSI cycles).

The study was designed to compare group A with group B, but results in the other groups were considered as well. The study was approved by the Institutional Ethical Board as well as by the sponsoring Foundation.

Ovarian stimulation protocol

Patients were treated with the long gonadotrophin releasing hormone (GnRH) agonist desensitization protocol, starting in the midluteal phase with $6 \times 100 \mu\text{g}$ of buserelin (Suprefact[®]; Hoechst, Frankfurt a/Main, Germany) intranasally for a period of 3 weeks. Gonadotrophin stimulation (Metrodin HP[®]; Serono, Geneva, Switzerland) was initiated if basal vaginal sonography showed a thin endometrium and no ovarian cysts. Stimulation was initiated with 150 IU of Metrodin HP[®], i.m. or s.c. except in patients with known poor response, where 225 IU was used.

The criterion for human chorionic gonadotrophin (HCG) administration was at least three mature follicles with a diameter of 18 mm HCG (Profasi[®]; Serono), 10 000 IU i.m. was given exactly 37 h before oocyte retrieval.

IVF/ICSI procedure

Motile spermatozoa were isolated from fresh semen in a two-step protocol. First the spermatozoa were centrifuged on a mini-Percoll gradient consisting of three discontinuous layers (55–70–90%). The 90% fraction was washed with Medi-Cult medium. The resulting sperm pellet was resuspended and subsequently pipetted into the ring of a migration-sedimentation tube (Jondet *et al.*, 1987).

Ovum retrieval was performed vaginally under ultrasound guidance with local anaesthesia and sedation with midazolam hydrochloride (Dormicum[®]; Roche, Brussels, Belgium). Follicles were flushed with HEPES-buffered Earle's balanced salt solution (EBSS; Bio-Whittaker/

Boehringer, Verviers, Belgium) containing 5 units/ml heparin and 1 mmol/l EDTA (Sigma, St Louis, MO, USA). Cumulus–oocyte complexes were isolated from the follicular aspirates, washed in Universal IVF medium (Medi-Cult, Copenhagen, Denmark), each placed individually in a 25 μ l microdrop of INRA–Ménézo B2 medium (CCD, Paris, France) under mineral oil (Sigma) and incubated at 37°C in a humidified atmosphere of 5% CO₂ in air.

For standard IVF, 3–5 h after retrieval every oocyte was inseminated with 20 000 motile spermatozoa and incubated overnight. The ICSI procedure was performed (Van Steirthegehem *et al.*, 1993a,b).

Approximately 16–19 h after insemination/injection normal fertilization was checked. All oocytes containing two clearly visible pronuclei were placed together in one fresh 10 μ l microdrop of Ménézo B2 medium (maximum 10 oocytes/drop) and cultured for another 24 h. The next day (40–43 h after insemination/injection) the embryos were separated, each being transferred to a 10 μ l drop of M3 Medium (Medi-Cult) for further culture of 24 h. Every embryo was scored for the total number of cells, the presence of anuclear fragments as well as multinucleated blastomeres.

On day 3 (64–67 h after insemination/injection) embryo quality was evaluated again. Selection for embryo replacement was made according to the top quality embryo selection criteria described by Van Royen *et al.* (Van Royen *et al.*, 1999).

Embryo transfer

All transfers were performed as an outpatient procedure as follows. The patient was placed in lithotomy position and the legs covered with sterile drapings. Sterile powder-free gloves were used throughout the procedure. A sterile speculum was introduced and the cervix gently exposed. Using sterile cotton wool, all visible vaginal contents were removed and the cervix cleansed with sterile saline. The external os of the cervix was wetted with a cotton stick drenched in buffered culture medium. All transfers were performed using a Wallace embryo transfer catheter (Sims Portex Ltd, Hythe, Kent, UK), consisting of an inner catheter and an outer catheter showing a calibration in centimetres. The outer catheter was introduced first, using a guidewire which allowed the outer catheter to be bent if necessary in order to facilitate its passage through the cervical channel. Care was taken to limit the introduction of the outer catheter to a maximum of 3–4 cm into the cervix, in order to minimize potential microtrauma of the uterine cavity. The inner catheter was then passed through the outer catheter, passing the external ostium by a distance of 6 cm. The embryo(s) were then gently deposited into the cavity in a volume <30 μ l of Medi-Cult M3 medium.

After removal, the catheter was checked under the microscope to ascertain that the embryos were left behind into the uterine cavity. In cases where the introduction of the outer supporting catheter proved impossible, a tenaculum was placed on the anterior lip of the cervix and the cervix was stretched, allowing the catheter to be introduced. After this procedure, all patients were requested to rest for 1 h in a hospital bed.

Luteal phase

In all cycles, luteal phase was supported with 3 \times 200 mg of micronized natural progesterone (Utrogestan®; Laboratoires Piette International, Brussels, Belgium), administered vaginally. A blood sample was taken on day 8 and 12 after embryo transfer for analysis of serum oestradiol, progesterone and HCG concentrations.

A cycle was considered a conception cycle when two rising HCG values of >5 mIU/ml were subsequently obtained on day +12 after transfer and any day thereafter. A clinical miscarriage was defined as a conception cycle leading to loss of at least one amniotic sac identified at sonography, or a clinical extrauterine pregnancy

necessitating a surgical intervention. An ongoing pregnancy was defined as a conception cycle with at least one fetal sac with a positive heart beat beyond 12 weeks of amenorrhoea. For the calculation of the implantation rate, biochemical pregnancies were not included, while a clinical miscarriage or a clinical extrauterine pregnancy counted as one implantation.

Results were expressed using confidence interval (CI) analysis with 95% confidence limits.

Results

Results are shown in Table I.

The implantation rate (IR) was the same in the single embryo transfer and double embryo transfer groups [42.3 and 48.1% respectively; relative risk (RR) = 0.88, 95% CI = 0.52–1.49].

The ongoing pregnancy rate (OPR) was higher in the double embryo transfer group than in the single embryo transfer group (74.1 versus 38.5% respectively; RR = 1.75; 95% CI = 1.06–2.89). However, the OPR in the single embryo transfer group was still equal to or higher than the generally accepted monthly fecundity rate of a couple with normal fertility. When all single embryo transfer cycles (including the elective single embryo transfers) were considered together, the OPR was 15/37 = 40.5%. There was one monozygotic twin in the single embryo transfer group (A) versus 6/20 = 30% (dizygotic) twins in the double embryo transfer group (B).

The OPR in the double embryo transfer group was also higher than in group C (RR = 1.78; 99% CI = 1.07–2.98). Similarly, the IR was higher in the double embryo transfer group (48.1%) as compared to group C (30.8%; RR = 1.56; 95% CI = 1.05–2.32), group E (31.8%; RR = 1.51; 95% CI = 1.04–2.20) and group F (16.7%; RR = 2.88; 95% CI = 2.10–3.95). The dizygotic twin pregnancy rate was similar in all patients belonging to groups B (31.6%), C (50%), E (37.9%) and F (35.8%). There were two dizygotic triplets, one in group E and one in group F.

Discussion

In the human, twin and high order multiple pregnancies are not physiological. Thus, if resulting from artificial reproductive technology, they are to be considered iatrogenic complications.

Until now, a number of preventive strategies have been considered in order to reduce the risk of multiple births. Although efforts to adjust the number of embryos transferred to limit the risk of multiple pregnancy, while maintaining an acceptable pregnancy rate, have resulted in some reduction in the incidence of high order multiple pregnancies, they have failed to decrease substantially the incidence of twin pregnancy. These efforts and recommendations include: replacing three embryos only in the group aged 38–40 years and over (van Kooij *et al.*, 1996), limiting the number of embryos transferred to a maximum of two (Erenus *et al.*, 1991; Waterstone *et al.*, 1991; Staessen *et al.*, 1992, 1993; Steer *et al.*, 1992; Visser and le Fourie, 1993; Shulman *et al.*, 1993; Lewin *et al.*, 1994; Roest *et al.*, 1997; Hu *et al.*, 1998), prolonging the culture of the embryos *in vitro* (Huisman *et al.*, 1994; Scholtes and Zeilmaker, 1996; Abdelmassih *et al.*, 1998), and culturing

Table I. Comparative outcome of six groups of patients: group A ($n = 26$; single embryo transfer), group B ($n = 27$; double embryo transfer), group C ($n = 57$; recruited, did not produce two top quality embryos but received all two best embryos except three patients), group D1 ($n = 11$; elective single embryo transfer, received one top embryo), group D2 ($n = 6$, elective single embryo transfer, did not receive one top embryo), group E ($n = 67$; did not agree to participate) and group F ($n = 352$; all other cycles, not recruitable by inclusion criteria)

	A Single embryo transfer	B Double embryo transfer	C NR	D1 eSingle embryo transfer+	D2 eSingle embryo transfer-	E NA	F NE
No. of cycles (transfers)	26	27	57 (53)	11	6 (4)	67 (64)	352 (328)
Mean no. of embryos replaced	1	2	1.81	1	1.2	1.93	2.5
Positive HCG	17	22	25	6	0	37	143
Clinical pregnancies	14	21	24	5	0	33	118
Ongoing pregnancies	10	20	22	5	0	29	95
Multiple pregnancies	1 ^a	6	11	0	0	11 ^b	34 ^b
Conception rate (%)	65.4	81.5	47.2	54.5	0	57.8	43.6
Clinical PR (%)	53.8	77.7	45.3	45.5	0	51.6	36.0
Ongoing PR (%)	38.5	74.1	41.5	45.5	0	45.3	29.0
Multiple PR (%)	10.0	6/20 (30)	11/22 (50)	0	0	11/29 (37.9)	34/95 (35.8)
Implantation rate (%)	11/26 (42.3)	26/54 (48.1)	33/107 (30.8)	5/11 (45.5)	0	41/129 (31.8)	136/813 (16.7)

^aOne monozygotic twin.

^bTwo dizygotic triplets.

NR = not randomizable; eSingle embryo transfer+ = requested single embryo transfer and received one top quality embryo; eSingle embryo transfer- = requested single embryo transfer and did not receive one top quality embryo; NA = not agreed to participate; NE = not eligible; HCG = human chorionic gonadotrophin.

embryos to day 5 or longer so as to transfer blastocysts rather than embryos during their early cleavage stages (Bavister and Boatman, 1997; Desai *et al.*, 1997; Gardner and Lane, 1997; Gardner *et al.*, 1998a; Jones *et al.*, 1998; Balaban *et al.*, 1998; Pantos *et al.*, 1998). The only existing prospective randomized study compared transfer of four versus two embryos in fresh IVF cycles (Vauthier-Brouzes *et al.*, 1994).

None of these studies has offered a solution to avoid twin pregnancies.

Transfer of one single embryo as a standard routine for the majority of IVF/ICSI cycles has been theoretically considered but not practised. The potential beneficial effect on the multiple pregnancy rate has been estimated on the basis of a retrospective analysis (Coetsier and Dhont, 1998). It has been stated that the wider the definition of the high risk group, the greater the impact on the multiple pregnancy rate but also the greater the impact on the overall pregnancy rate, and vice versa. Defining an acceptable multiple pregnancy rate will always remain a matter of clinical judgement. We started from the point of view that a twin pregnancy rate of around its spontaneous incidence of ~1% will be acceptable to all.

Ideally, if only single embryo transfer were performed, the number of multiples would be almost as low as in natural conception cycles. A generalized introduction of single embryo transfer was until now not a serious option because it was feared that the overall success rate of IVF would drop too low. This has been demonstrated by results of (mostly compulsory) single embryo transfer, where only one embryo, often of poor quality, was available to be replaced (ASRM/SART Registry, 1995 and 1996; Austin *et al.*, 1996; Svendsen *et al.*, 1996; Walters, 1996), yielding a pregnancy rate of <10% per started cycle. A French retrospective study (Giorgetti

et al., 1995) of 957 compulsory single embryo transfer showed that the implantation rate went up from roughly 4% per transfer for poor embryos to 8% for fair, 12% for good and 16% for excellent embryos. The latter value corresponds to that found in the only report on elective single embryo transfer until now: in this Finnish study (Vilksa *et al.*, 1998), 169 elective embryo transfers were performed of one embryo in 112 standard IVF cycles and 57 ICSI cycles. In patients who had only one embryo available ($n = 95$), they obtained a pregnancy rate of 20% (19/95); in patients who had an elective single embryo transfer, they obtained an excellent pregnancy rate of 29.8% (22/74). However, elective single embryo transfer was not performed in this study with an aim to decrease the multiple pregnancy rate in the general assisted reproductive treatment population, but because these were patients with compelling medical reasons to avoid multiple pregnancy, e.g. diabetes mellitus, uterine malformation, history of cervical incompetence or hysterotomy, or indication for prenatal diagnosis. Therefore, these single embryo transfer should be termed medical single embryo transfer rather than elective single embryo transfer. Nevertheless, these results give an idea of what can be expected of single embryo transfer as a routine policy in a selected group of patients.

This study compared prospectively two groups of patients, who were comparable in every respect, all patients having at their disposal two top quality embryos, half of whom were randomized to receive one embryo and the other half two. It is essential to be able to distinguish embryos with high implantation potential as early as possible. We have used characteristics of a top quality embryo in our study which were not arbitrarily chosen but were based on identification of objective and strict criteria and have been validated in a

retrospective study prior to this prospective trial (Van Royen *et al.*, 1999). These results show that it is possible to detect embryos with a very high clinical and ongoing implantation potential (± 45 and $\pm 40\%$ respectively). Although the ongoing pregnancy rate after double embryo transfer of two top embryos can be high (74% in our study), it is inevitably linked to an unacceptable rate of twin pregnancies, whereas transfer of one top quality embryo can yield an ongoing pregnancy rate of $>30\%$, which should be acceptable to all parties involved in IVF, and is not accompanied by the risk of dizygotic twin pregnancy. The data from our study provide an important instrument in the prevention of twins, especially in the group most at risk for twins, i.e. patients during their first trial and who are <34 years of age. In our own programme, $>90\%$ of all twins occur in the first two IVF cycles.

In the minds of many women, a twin pregnancy is a desirable goal. A great deal of counselling was required to convince the patients and their husbands of the need for a cautious embryo replacement strategy and to participate in the study. We also had to take care that the patients should not interpret the study design as a choice offered to them between single embryo transfer and double embryo transfer, but as a choice to participate or not to participate in a study with blind random allocation.

It is noteworthy that 67/194 (34.5%) of the patients who complied with the clinical inclusion criteria (<34 years of age, first IVF cycle) did not agree to participate in the study. This was due to one of the following reasons: firm belief that they would jeopardize their chance of obtaining success by transferring only one embryo; fear of a twin pregnancy leading to a request for elective single embryo transfer; patients who did not receive counselling due to lack of time; patients who for ethical reasons, e.g. repeated miscarriages in their history, could not be asked to participate in a study protocol; or patients who for other reasons could not be recruited, e.g. language barrier or foreign patients who could only have one single trial.

To understand the similar percentages of twin pregnancies in all groups, two considerations must be made. First, although the implantation rate was lower in groups C, E and F than in groups A, B and D, the number of embryos transferred was higher. Secondly, on the basis of the numbers in this trial, it appears that adding one non-top quality embryo to one top quality embryo still creates an undiminished risk for a twin pregnancy as compared to transferring two top quality embryos. Application of strict embryo criteria therefore does not lead to twin pregnancy prevention unless it is decided to transfer only one single (top quality) embryo.

We have tried to assess the size of our IVF/ICSI population which could potentially benefit from the single embryo transfer strategy as a prevention of twin pregnancy. This is illustrated in Table II: overall 371/512 (72.5%) of all embryo transfers concerned at least one top quality embryo, in which the principle of twin prevention by performing single embryo transfer of one top quality embryo was applicable.

A further analysis of our data, including all women up to ≤ 38 years, shows that all conclusions remain valid, even including the age group 34 to <38 years of age.

Looking at parameters of embryo polarity, pronuclear rota-

Table II. Total potential target population for twin prevention: proportion of embryo transfers with at least one top quality embryo

Maternal age (years)	No. of embryos/transfer	No. of transfers	No. of transfers with at least one top quality embryo	% of total
<34	1	54	44	81.5
	2	228	164	71.9
	total	282	208	73.8
<38	1	76	57	75.0
	2	290	212	73.1
	total	366	169	73.5
<38	>2	146	102	70.0
	total	512	371	72.5

tion or the segregation of polarized proteins into blastomeres have been suggested as alternatives for the early detection of embryos with potentially high implantation rates (Edwards and Beard, 1999). Scott and Smith (1998) have described a scoring system based on a number of morphological characteristics of pronuclear stage embryos. Their highest ranking embryos reached an implantation rate of 28%, comparable to our evaluation method. When the results of Scott and Smith (1998) were compared with those obtained with blastocyst stage transfer (Gardner *et al.*, 1998b), the implantation rate of blastocysts, corrected for the number of embryos lost (46.5%), was only 21%. Moreover, the highest multiple pregnancy rate was obtained in the blastocyst transfer group (reviewed by Edwards and Beard, 1999). All these efforts do not lead to an elimination of twin pregnancies, unless they are combined with single day 3 embryo or perhaps single blastocyst transfer. We think our criteria are easier to assess, do not necessitate the laborious culture of embryos to the blastocyst stage, and in our hands they are applicable to 72.5% of all cycles and they have been validated both in a retrospective study (Van Royen *et al.*, 1999) and in this prospective study in which single embryo transfer was applied as the unavoidable complementary tool to optimal embryo selection.

We believe that patients achieving at least one top quality embryo should be offered single embryo transfer for at least one treatment cycle, probably more. The concept of single embryo transfer being efficacious in first treatment cycles, it could be applied in a cumulative fashion, e.g. during the first two or three treatment cycles. This should be evaluated in a life-table fashion. It is probable that the same total proportion of patients will eventually obtain a (singleton) pregnancy as after double embryo transfer, but probably after a slightly larger number of treatment cycles.

There are other interesting aspects to a systematic single embryo transfer strategy.

First, if the habit to transfer fewer embryos, i.e. preferably just one if of sufficient prospective viability, became the standard of good medical practice, clinicians could start working out ovarian stimulation protocols which are less aggressive than those presently used. The time to revolutionize ovarian stimulation, as proposed by leading experts (Edwards *et al.*, 1996), will then be matched by an appropriate embryo replacement strategy. The savings obtained by a dramatic reduction in IVF twin numbers could then be made available

for IVF treatment, until now unrefunded in most countries. An effort has been made to compare the hypothetical cost for society incurred by pregnancies achieved with IVF protocols based on actual (for two-embryo transfers) and hypothetical (for one-embryo transfers) take-home baby rates in Sweden (Wølner-Hanssen and Rydhstroem, 1998). This has shown that single embryo transfer is cost-saving while still guaranteeing similar cumulative pregnancy chances to patients on a 'one-at-a-time' basis.

Secondly, the single embryo transfer concept could also be applied in cryotransfers, especially in women who have quickly become pregnant during their fresh IVF treatment. It should certainly be used in all medical indications to avoid twins, e.g. those mentioned earlier, and in a number of other indications, e.g. women who already have children, single women or women requesting donor insemination in a stimulated cycle, etc.

Thirdly, the hypothesis that the expensive and work-intensive prolonged culture of blastocysts will result in a high pregnancy rate while lowering the incidence of twins has to be checked in a prospective study, comparing transfer of one top quality embryo with transfer of one blastocyst. Until such data are available, and if, when obtained, they fail to show any benefit from single blastocyst transfer as compared to single embryo transfer, blastocyst culture as a solution for the twin problem would remain highly questionable.

Finally, in order to switch to single embryo transfer as the standard of good medical practice in new IVF patients, a change in attitude is necessary both in patients and in doctors. It can only work if all the major centres practise single embryo transfer, but not if some continue to replace two or more embryos. To make this happen, fertility centres around the world, and on a national level, should, by a mechanism of peer review, make sure that single embryo transfer is accepted as routine policy in all centres.

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